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APPLICATION NUMBER: 60/526,977

FILING DATE: December 05, 2003

By Authority of the  
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*T. Lawrence*

T. LAWRENCE  
Certifying Officer

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**PROVISIONAL APPLICATION FOR PATENT COVER SHEET**

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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60/526977

120503

| INVENTOR(S)                                                                                                                                              |  |                        |  |                                                         |  |
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| <input type="checkbox"/> Additional inventors are being named on the _____ separately numbered sheets attached hereto                                    |  |                        |  |                                                         |  |
| TITLE OF THE INVENTION (500 characters max)                                                                                                              |  |                        |  |                                                         |  |
| IMMUNOLOGIC COMPOUNDS FOR PREVENTION, PROTECTION, PROPHYLAXIS OR TREATMENT OF IMMUNOLOGICAL DISORDERS, INFECTIONS AND CANCER                             |  |                        |  |                                                         |  |
| Direct all correspondence to: CORRESPONDENCE ADDRESS                                                                                                     |  |                        |  |                                                         |  |
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| ENCLOSED APPLICATION PARTS (check all that apply)                                                                                                        |  |                        |  |                                                         |  |
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| <input type="checkbox"/> Application Data Sheet, See 37 CFR 1.76                                                                                         |  |                        |  |                                                         |  |
| METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT                                                                             |  |                        |  |                                                         |  |
| <input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27                                                                           |  |                        |  | FILING FEE AMOUNT (\$)                                  |  |
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| The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.                  |  |                        |  |                                                         |  |
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| <input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are: _____                                       |  |                        |  |                                                         |  |

Respectfully submitted,

SIGNATURE

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Date

December 5, 2003

REGISTRATION NO.  
If appropriate)

37,037

Docket Number:

15922-3USPR FC

- 1 -

**IMMUNOLOGIC COMPOUNDS FOR PREVENTION,  
PROTECTION, PROPHYLAXIS OR TREATMENT OF  
IMMUNOLOGICAL DISORDERS, INFECTIONS AND  
CANCER**

**5    BACKGROUND OF THE INVENTION**

**(a) Field of the invention**

The present invention relates to the use of PDT-treated cells (whole or fragments thereof) and/or supernatant thereof in the preparation of vaccines for immunoprotection or immunomodulation. The PDT-treated cells and lysate  
10    thereof are prepared by treating cells with photoactivatable molecules, such as 2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)-benzoic acid methyl ester hydrobromide (hereinafter referred to as TH9402) and derivatives thereof (as described in International Patent Application published under No. WO 02/079183) and activating these molecules with light. The main characteristic of these  
15    molecules is their ability to accumulate into and eradicate cells once activated, such cells include, without limitation, immune cells, cancer cells, infected cells, without affecting progenitor stem cells. This particularity is of great interest since repetitive extracorporeal treatment of blood cells and their reinjection into the bloodstream has a limited effect on lymphocytes, as mainly activated cells will be  
20    eradicated, and resting cells are spared in higher proportions. While intercalating agent such as 8-methoxypsoralen necessitate the usage of UV irradiation, photoactivatable molecules of the present invention (TH9402 and derivatives thereof) use visible light for their activation, thereby reducing the risk of mutagenic effects. Other agents such as Intercept<sup>TM</sup> are also intercalating agents.  
25    Since the photoactivatable molecules of the present invention (TH9402 and derivatives thereof) do not accumulate in cell nuclei, they have a low potential of causing DNA damage, mutation and/or carcinogenesis.

**(b) Brief description of the prior art**

Photodynamic Therapy (PDT) uses chemical compounds activated by  
30    various light/irradiation devices. Cytotoxicity of the activated species leads to the eradication of cells. Photodynamic therapy could also affect targeted cells by

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inducing apoptosis. Apoptotic cells are known for their capacity to present their own antigens to professional antigen presenting cells, such as dendritic cells. Such antigen presentation can lead to the development of a response of the immune system toward these immunizing antigens. Many reports have demonstrated the usefulness of adjuvants to boost the immune response toward the killed cells. Among others, pertinent references such as works by Korbelik (Korbelik *et al*, *Laser Med. Surg*, 14 (1996), 329-334, *Can. Res.*, 56, (1996) 5647-5565; Chen *et al*, *SPIE*, 394 (2000), 26-32), as well as Nordquist *et al* (International Patent Applications published under Nos. WO 96/31237 and WO 99/47162A1) have demonstrated the usefulness of such an approach. Moreover, the usage of oxygenated species in blood components has been described previously using ozone as the chemical agent in conjunction with irradiation (Zee *et al*, US Patent No. 4,632,980; Fish *et al*, US Patent No. 4,831,268, Mueller *et al*, US Patent No. 4,968,483). Photodynamic Therapy has also been extensively described in "Photosensitizing Compounds: their Chemistry, Biology and Clinical uses" (1989, John Wiley & Sons, Chichester, UK, ISBN 0471923087). Many other pertaining references relating to the usage of Photosensitizers in the treatment of tumor masses combined with antibodies (Levy *et al*, US Patents Nos. 5,095,030 & 5,283,225) as well as ligands and antibodies (Pendry *et al*, US Patent No. 5,241,036). Autoimmune vaccines have been described by Bolton, A.E. (US Patent No. 6,204,058B1) (International Patent Application published under No. WO 98/07436) on which Rheumatoid Arthritis is treated using leukocytes with increased expression of specific antigens by oxidizing agents, UV irradiation and high temperature.

Extracorporeal Photopheresis has been described as a successful therapy for the treatment of Hepatitis C, in combination with other means such as Interferon alpha (O'Brien, C.B. International Patent Application published under No. WO 97/376542; McLaughlin S.N. *et al*, International Patent Application published under No. WO 97/36634), as well as in the treatment of other illnesses mediated by undesired activated immune cells (McLaughlin *et al*, US Patent No. 5,984,887 and Bisaccia *et al*, US Patent No. 5,426,116). Other studies have been

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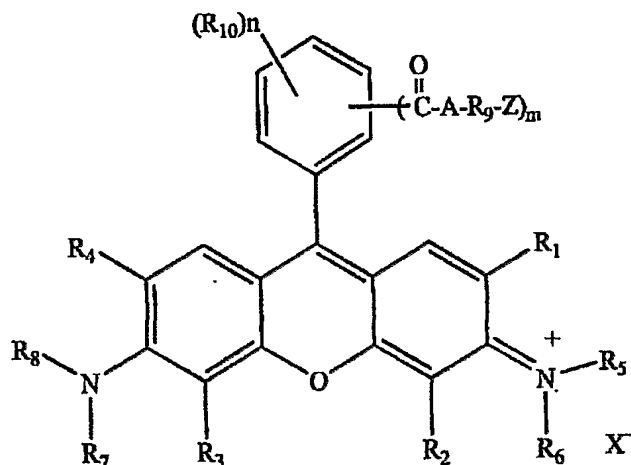
reported regarding the usage of extracorporeal Photopheresis in indications such as organ rejection (Lehrer et al, 2001, *The journal of Heart and Lung transplantation*, November, 1133-1136; Rosa et al, 1992, *Transplantation*, 4(53), 808-815; Barr et al, 1998, *The New England Journal of Medicine*, 339(4), 1744-1751, Barr et al, 2000, *Clinical Transplantation*, 14, 162-166) as well as for the efficacious treatment of Graft-versus-Host-Disease (Perotti et al, 1999, *Haematologica*, 84, 237-241; Amico et al, 1997, *British Journal of Hematology*, 97, 848-854; Rossetti et al, 1995, *Transplantation*, 59(1), 149-151; Gorgun et al, 2002, *Immunobiology*, 100(3), 941-947). The indications for the usage of extracorporeal Photopheresis is reviewed by Ratanatharathorn et al. in *Bone Marrow Transplantation* (2001, 28, 121-129).

It would be highly desirable to be provided with the use of PDT-treated cells and supernatant thereof in the preparation of immunologic compounds for prevention, protection, prophylaxis or treatment of immunological disorders, infections and/or a cancers in an individual

#### **SUMMARY OF THE INVENTION**

In accordance with the present invention, there is provided the use of PDT-treated cells (whole or fragments thereof) and/or supernatant thereof in the preparation of an immunologic compound for prevention, protection, prophylaxis or treatment of an immunological disorder, infection and/or a cancer in an individual, which comprises treatment of said individual cells or components thereof with a photoactivatable molecule of formula (I):

- 4 -



(I)

wherein:

- 5       - one of  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ , and  $R_{10}$  represents an halogen atom and each of the remaining  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ , and each of the remaining  $R_{10}$  group is independently selected in the group constituted by hydrogen, halogen atoms, an amino, acylamino, dialkylamino, cycloalkylamino, azacycloalkyl, alkylcycloalkylamino, aroylamino, diarylamino, arylalkylamino, aralkylamino, alkylaralkylamino, arylaralkylamino,
- 10       hydroxy, alkoxy, aryloxy, aralkyloxy, mercapto, alkylthio, arylthio, aralkylthio, carboxyl, alkoxycarbonyl, aryloxy carbonyl, aralkoxycarbonyl, carbamoyl, alkylcarbamoyl, dialkylcarbamoyl, cyano, hydroxysulfonyl, amidosulfonyl, dialkylamid sulfonyl, arylalkylamid sulfonyl, formyl, acyl, aroyl, alkyl, alkylene, alkenyl,
- 15       aryl, aralkyl, vinyl, alkynyl group and by the corresponding substituted groups;
- $m = 0 - 1$ ;
- $n = 1 - 4$ ;
- A is nil, O, or NH;
- 20       -  $R_9$  represents an alkylene group;
- Z is H, amino, dialkylamino, or trialkylamino salt;
- $X^-$  is an anion; and

- 5 -

- R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub> and R<sub>8</sub> are independently H or C<sub>1</sub>-C<sub>6</sub> alkyl or R<sub>1</sub> in combination with R<sub>5</sub> or R<sub>6</sub>, or R<sub>2</sub> in combination with R<sub>5</sub> or R<sub>6</sub>, or R<sub>3</sub> in combination with R<sub>7</sub> or R<sub>8</sub>, or R<sub>4</sub> in combination with R<sub>7</sub> or R<sub>8</sub> represents an alkylene,

5 and wherein said photoactivatable molecule is activated by a light of appropriate wavelength, thereby activating said photoactivatable molecule and causing prevention, protection, prophylaxis or treatment of said immunological disorder, infection and/or a cancer.

10 Also in accordance with the present invention, there is provided an immunologic vaccine comprising PDT-treated cells (whole or fragments thereof) and/or supernatant thereof, wherein said cells are treated with a photoactivatable molecule of formula (I) as previously defined, in association with a pharmaceutically acceptable carrier. The vaccine of the invention can be used for  
15 prevention, protection, prophylaxis or treatment of said immunological disorder, infection and/or a cancer.

Still in accordance with the present invention, there is provided a method of preparing an immunologic compound for prevention, protection, prophylaxis or  
20 treatment of an immunological disorder, infection and/or a cancer in an individual, which comprises the steps of :

- a) treatment of said individual cells with a photoactivatable molecule of formula (I) as previously defined; and  
25 b) subjecting said cells to a light of appropriate wavelength to activate said photoactivatable molecule, thereby obtaining PDT-treated individual cells (whole or fragments thereof) and/or supernatant thereof.

30 For the purpose of the present invention the following terms are defined below.



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"PDT-treated cells" means cells which have been treated with a photoactivatable molecule activated by a light of appropriate wavelength;

- 5        - "alkyl" means a straight or branched aliphatic hydrocarbon group and the corresponding substituted alkyl group bearing one or more substituents which may be the same or different and which are selected in the group constituted by halo, aryl, hydroxy, alkoxy, aryloxy, alkyloxy, alkylthio, arylthio, aralkyloxy, aralkylthio, and cycloalkyl and "branched" means that a lower alkyl group such as methyl, ethyl or propyl is attached to a linear alkyl chain, preferred alkyl groups include the "lower alkyl" groups which are those alkyl groups having from about 1 to about 6 carbons., exemplary alkyl groups are methyl, ethyl, isopropyl, hexyl, cyclohexylmethyl, methyl or ethyl groups are more preferred;
- 10        - "cycloalkyl" means a non-aromatic ring preferably composed from 4 to 10 carbon atoms, and the cyclic alkyl may be partially unsaturated, preferred cyclic alkyl rings include cyclopentyl, cyclohexyl, cycloheptyl, the cycloalkyl group may be optionally substituted with an aryl group substituent, the cyclopentyl and the cyclohexyl groups are preferred;
- 15        - "alkenyl" means an alkyl group containing a carbon-carbon double bond and having preferably from 2 to 5 carbon atoms in the linear chain, exemplary groups include allyl vinyl;
- 20        - "alkynyl" means an alkyl group containing a carbon-carbon triple bond and having preferably from 2 to 5 carbon atoms in the linear chain, exemplary groups include ethynyl, propargyl;
- 25        - "aryl" means an aromatic carbocyclic radical or a substituted carbocyclic radical containing preferably from 6 to 10 carbon atoms, such as phenyl or naphthyl or phenyl or naphthyl substituted
- 30

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by at least one of the substituents selected in the the group constituted by alkyl, alkenyl, alkynyl, aryl, aralkyl, hydroxy, alkoxy, aryloxy, aralkoxy, carboxy, aroyl, halo, nitro, trihalomethyl, cyano, alkoxycarbonyl, aryloxycarbonyl, aralkoxycarbonyl, acylamino, aroylamino, carbamoyl, alkylcarbamoyl, dialkylcarbamoyl, alkylthio, arylthio, alkylene or -NYY' where Y and Y' are independently hydrogen, alkyl, aryl, or aralkyl;

- "aralkyl" means a radical in which an aryl group is substituted for an alkyl H atom, exemplary aralkyl group is benzyl;

- "acyl" means an alkyl-CO- group in which the alkyl group is as previously described, preferred acyl have an alkyl containing from 1 to 3 carbon atoms in the alkyl group, exemplary groups include acetyl, propanoyl, 2-methylpropanoyl, butanoyl or palmitoyl;

- "aroyl" means an aryl-CO- group in which the aryl group is as previously described and preferably contains from 6 to 10 carbon atoms in the ring, exemplary groups include benzoyl and 1- and 2-naphtoyl;

- "alkoxy" means an alkyl-O- group in which the alkyl group is as previously described, exemplary alkoxy groups include methoxy, ethoxy, n-propoxy, i-propoxy, n-butoxy, and heptoxy;

- "aryloxy" means an aryl-O- group in which the aryl group is as previously described, exemplary aryloxy groups include phenoxy and naphthoxy;

- "alkylthio" means an alkyl-S-group in which the alkyl group is as previously described, exemplary alkylthio groups include methylthio, ethylthio, i-propylthio and heptylthio;

- "arylthio" means an aryl-S-group in which the aryl group is as previously described, exemplary arylthio groups include phenylthio, naphthylthio;

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- "aralkyloxy" means an aralkyl-O- group in which the aralkyl group is as previously described, exemplary aralkyloxy group is benzyloxy;
- 5 - "aralkylthio" means an aralkyl-S- group in which the aralkyl group is as previously described, exemplary aralkylthio group is benzylthio;
- "dialkylamino" means an -NYY' group wherein both Y and Y' are alkyl groups as previously described, exemplary alkylamino include ethylamino, dimethylamino and diethylamino;
- 10 - "alkoxycarbonyl" means an alkyl-O-CO- group wherein the alkyl group is as previously described, exemplary alkoxycarbonyl groups include methoxy- and ethoxy-carbonyl;
- "aryloxycarbonyl" means an aryl-O-CO- group wherein the aryl group is as previously described, exemplary aryloxycarbonyl groups include phenoxy- and naphthoxy-carbonyl;
- 15 - "aralkoxycarbonyl" means an aralkyl-O-CO- group wherein the aralkyl is as previously defined, exemplary aralkoxycarbonyl group is benzyloxycarbonyl;
- "carbamoyl" is an H<sub>2</sub>N-CO- group;
- 20 - "alkylcarbamoyl" is an Y'YN-CO- group wherein one of Y and Y' is hydrogen and the other of Y and Y' is alkyl as defined previously;
- "dialkylcarbamoyl" is an Y'YN-CO- group wherein both Y and Y' are alkyl as defined previously;
- 25 - "acylamino" is an acyl-NH group wherein acyl is as defined previously;
- "aroylamino" is an aroyl-NH group wherein aroyl is as defined previously;
- 30 - "alkylene" means a straight or branched bivalent hydrocarbon chain group having preferably from 2 to 8 carbon atoms, and the alkylene group may be interrupted by one or more substituted

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nitrogen atoms wherein the substituent of the nitrogen atom is alkyl or oxygen or sulfur atoms, and it is presently more preferred that the alkylene group has from 2 to 3 carbon atoms, exemplary alkylene groups include ethylene ( $-\text{CH}_2\text{CH}_2-$ ), propylene ( $-\text{CH}_2\text{CH}_2\text{CH}_2-$ ),  $-\text{CH}_2\text{NMe}-\text{CH}_2-$ ,  $\text{O}-\text{CH}_2-\text{O}$  or  $-\text{O}-\text{CH}_2\text{CH}_2-\text{O}-$ ;

- "halo" preferably means fluoro, chloro, bromo or iodo;

- "azacycloalkyl" preferably means a 4 to 9 membered saturated carbon ring where one of the methylene groups is replaced by nitrogen;

- "cycloalkylamine" means an  $-\text{NYY}'$  group wherein one of the Y and Y' is hydrogen and the other Y and Y' is cycloalkyl as defined previously;

- "alkylcycloalkylamino" means an  $-\text{NYY}'$  group wherein one of the Y and Y' is alkyl as defined previously and the other Y and Y' is cycloalkyl as defined previously;

- "diarylamine" means an  $-\text{NYY}'$  group wherein both Y and Y' are aryl groups as previously described;

- "aralkylamine" means an  $-\text{NYY}'$  group wherein one of the Y and Y' is hydrogen and the other Y and Y' is aralkyl as defined previously;

- "arylalkylamine" means an  $-\text{NYY}'$  group wherein one of the Y and Y' is alkyl as defined previously and the other Y and Y' is aryl as defined previously;

- "alkylaralkylamine" means an  $-\text{NYY}'$  group wherein one of the Y and Y' is alkyl as defined previously and the other Y and Y' is aralkyl as defined previously;

- "arylalalkylamine" means an  $-\text{NYY}'$  group wherein one of the Y and Y' is aryl as defined previously and the other Y and Y' is aralkyl as defined previously;

- 10 -

- "mercapto" is a -SH or a SR group wherein R may be any of the above defined groups  $R_1$  to  $R_{10}$ , the -SH, the mercaptoaryl and the mercaptoalkyl groups are preferred;

- "hydroxysulfonyl" is an  $-\text{SO}_3\text{H}$ ;

5 - "amidosulfonyl" is an  $-\text{SO}_2\text{NH}_2$ ;

- "dialkylamidosulfonyl" means an  $-\text{SO}_2\text{NYY}'$  group wherein both Y and Y' are alkyl groups as previously described;

10 - "arylalkylamidosulfonyl" means an  $-\text{SO}_2\text{NYY}'$  group wherein one of the Y and Y' is aryl as defined previously and the other Y and Y' is aralkyl as defined previously; and

15 - "anion" means the deprotonated form of an organic or inorganic acid and the anion is preferably selected from hydrochlorides, hydrobromides, sulfates, nitrates, borates, phosphates, oxalates, tartrates, maleates, citrates, acetates, ascorbates, succinates, benzenesulfonates, methanesulfonates, cyclohexanesulfonates, toluenesulfonates, sulfamates, lactates, malonates, ethanesulfonates, cyclohexylsulfamates, and quinate. In the case where the rhodamine derivative bears one or more acidsubstituents, the covered compound comprise the internal salt or any salt derived from neutralization by any of the following  
20 bases: sodium hydroxide, potassium hydroxide, calcium hydroxide, lithium hydroxide, ammonia, ethylene diamine, lysine, diethanolamine, piperazine and the like.

25

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

Fig. 1 illustrates a Kaplan-Meier survival analysis of mice after administration of cells treated or not with PDT.

30 Fig. 2 illustrates a tumor growth comparison between mice immunized with a supernatant from PDT-treated cells and mice which have not been immunized with such a supernatant.

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**DETAILED DESCRIPTION OF THE INVENTION****Immunological disorders, infections and cancers**

In the present invention, there is provided several solutions for prevention, protection and/or prophylaxis or treatment of an immunological disorder, infection and/or a cancer in an individual. In particular, the immunological disorder can be an alloimmune disorder or an autoimmune disorder. The alloimmune disorder can be Graft-versus-Host Disease or an organ rejection. Examples of autoimmune diseases include but are not limited to Rheumatoid Arthritis, Multiple Sclerosis, Scleroderma, Lupus, Autoimmune Hemolytic Anemia, Diabetes Mellitus, Progressive Systemic Sclerosis, Idiopathic Thrombocytopenic Purpura, Psoriasis, Ulcerative Colitis and Crohn's Disease. The infection can be caused by a bacteria, a virus, a parasite, a fungus, a prion, a protozoan or other infection agents. Also, the infection can cause Chagas' Disease. Examples of viruses include but are not limited to Human Immunodeficiency Virus (HIV), Hepatitis C Virus (HCV), Human Herpes Virus Type I or II, and Varicella Zoster. Example of cancers include but are not limited to solid tumors and hematologic tumors. The solid tumors can be of breast cancer, lung cancer, gastrointestinal cancer, skin cancer or of genitourinary, neurological, head and neck or musculoskeletal origin. The hematologic tumors can be lymphomas, leukemias, myelomas, myelodysplasias or plasma cell dyscrasias.

**Immunologic compounds of the present invention**

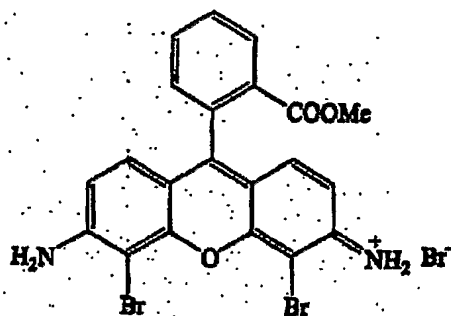
According to a preferred embodiment of the invention, the immunologic compounds include the photoactivatable molecules of formula (I) (or rhodamine derivatives) are molecules wherein at least two of the R1, R2, R3, R4, and R10 groups represent an halogen atom which is preferably a bromide atom. Preferably, the immunologic compounds of the invention are vaccines

30

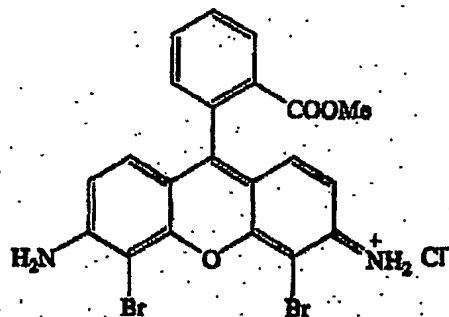
- 12 -

More preferred are the photoactivatable molecules wherein the halogen(s) atom is(are) on the 2-7, 4-5 or 4'-5' position on the ring or is(are) at the end of the ester chain.

5 The following specific photoactivatable molecules are particularly preferred:

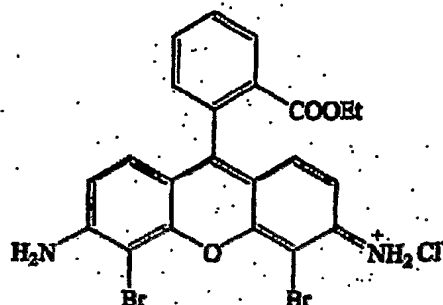


10 4,5-dibromorhodamine 123 hydrobromide (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)-benzoic acid methyl ester hydrobromide) also called TH9402,



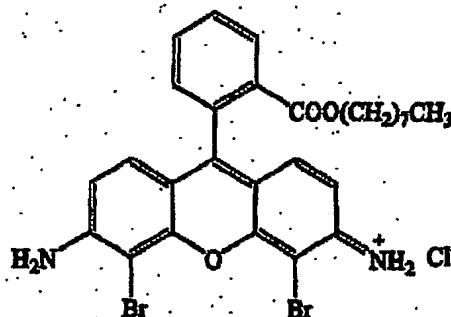
15 4,5-dibromorhodamine 123 hydrochloride (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)-benzoic acid methyl ester hydrochloride),

- 13 -



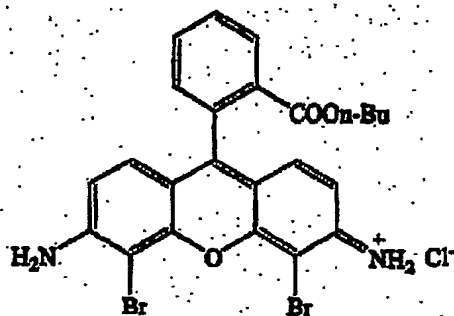
4,5-dibromorhodamine 110 ethyl ester hydrochloride (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid ethyl ester hydrochloride),

5



4,5-dibromorhodamine 110 octyl ester hydrochloride (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid octyl ester hydrochloride),

10

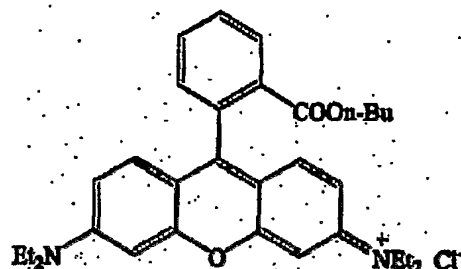


4,5-dibromorhodamine 110 n-butyl ester hydrochloride (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid n-butyl ester hydrochloride),

15

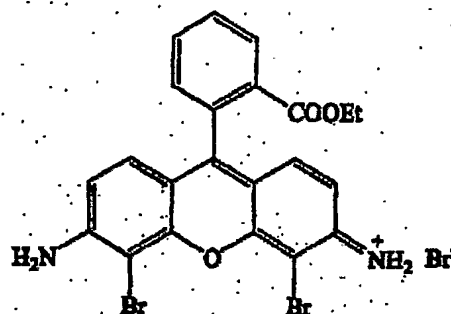


- 14 -



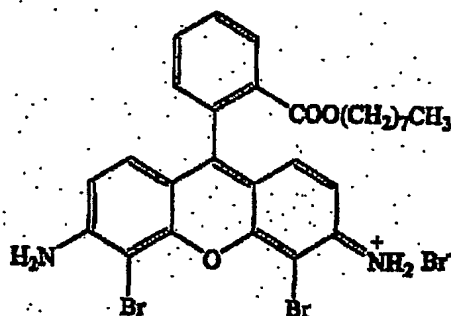
rhodamine B n-butyl ester hydrochloride (2'-(6-diethyl amino-3-diethyl imino-3H-xanthen-9-yl)benzoic acid n-butyl ester hydrochloride),

5



4,5-dibromorhodamine 110 ethyl ester hydrobromide (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid ethyl ester hydrobromide),

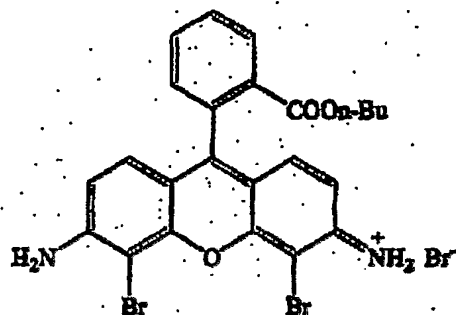
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4,5-dibromorhodamine 110 octyl ester hydrobromide (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid octyl ester hydrobromide),

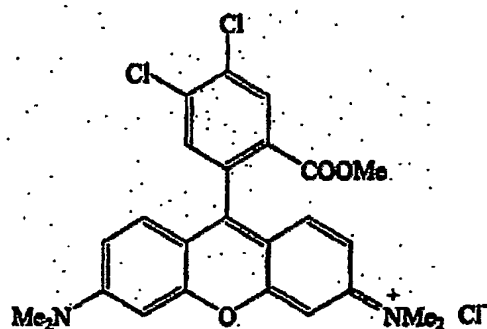
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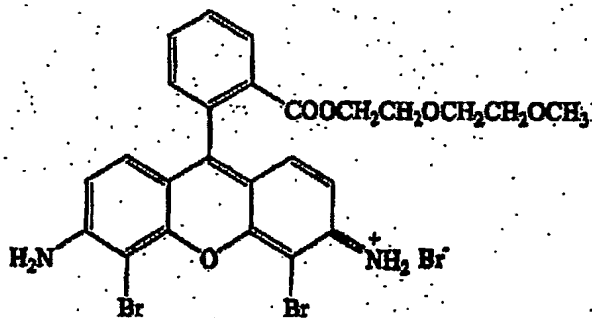
4,5-dibromorhodamine 110 n-butyl ester hydrobromide (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid n-butyl ester hydrobromide),

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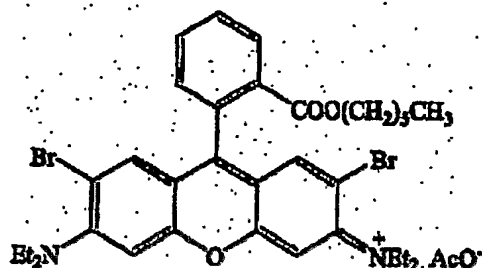
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10



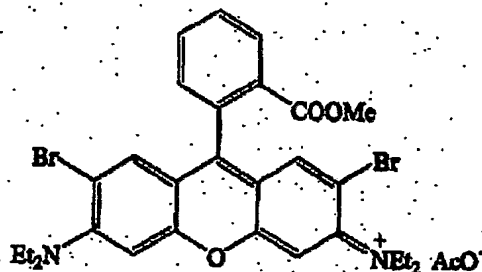
4,5-dibromorhodamine 110 2-(2-methoxy ethoxy)ethyl ester hydrobromide (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)-benzoic acid 2-(2-methoxy ethoxy) ethyl ester hydrobromide),

- 16 -



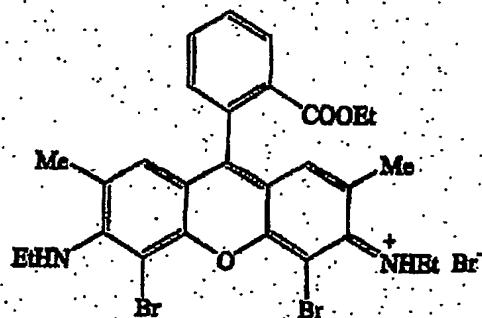
2,7-dibromorhodamine B hexyl ester acetate (2'-(2,7-dibromo-6-diethyl amino-3-diethyl imino-3H-xanthen-9-yl)benzoic acid hexyl ester acetate),

5



2,7-dibromorhodamine B methyl ester acetate (2'-(2,7-dibromo-6-diethyl amino-3-diethyl imino-3H-xanthen-9-yl)benzoic acid methyl ester acetate),

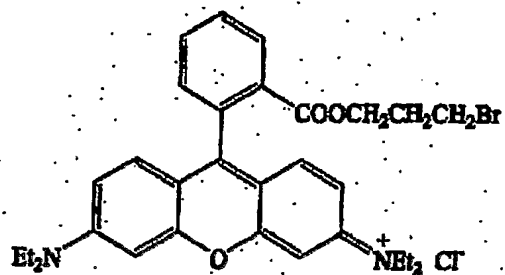
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4,5-dibromorhodamine 6G hydrobromide (2'-(4,5-dibromo-2,7-dimethyl-6-ethylamino-3-ethylimino-3H-xanthen-9-yl)benzoic acid ethyl ester hydrobromide),

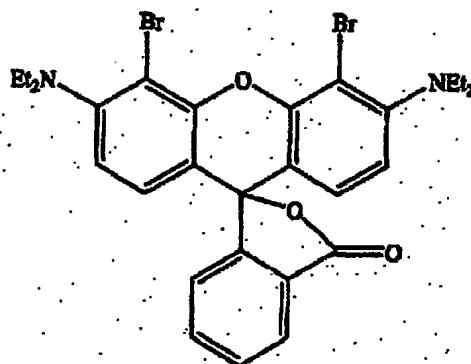
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- 17 -



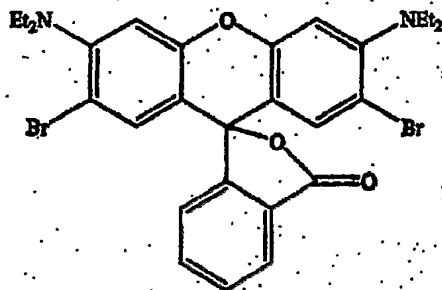
rhodamine B 3-bromopropylester hydrochloride (2'-(6-diethyl amino-3-diethyl imino-3H-xanthen-9-yl)benzoic acid 3-bromopropyl ester hydrochloride),

5



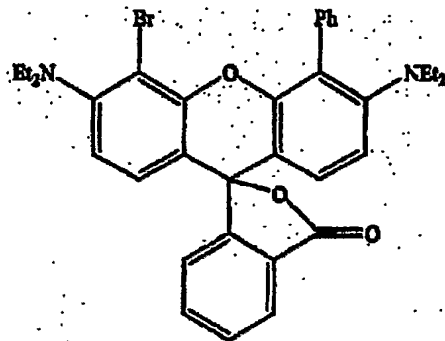
4,5-dibromorhodamine B base (3,3'-(4',5'-dibromo-3'-diethyl amino-6'-diethyl aminoxanthen-9'-yl)-3H-isobenzofuran-1-one),

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2,7-dibromorhodamine B base (3,3-(2',7'-dibromo-3'-diethyl amino-6'-diethyl aminoxanthen-9'-yl)-3H-isobenzofuran-1-one) and

5



4-bromo-7-phenyl-rhodamine B base (3,3-(4'-bromo-3'-diethyl amino-6'-diethyl amino-5'-phenyl xanthen-9'-yl)-3H-isobenzofuran-1-one).

10

The photoactivatable molecules of the invention are activatable by a light of appropriate wavelength which is preferably ranging from about 400 to about 800 nm and more preferably from about 450 to about 600 nm.

15

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### PDT of the present invention

PDT is preferably based on the exposition of the photoactivatable molecules of the invention to visible light, which can produce free radical species. Cationic rhodamines such as TH9402 have been shown to specifically accumulate  
5 in mitochondria and the production of free radicals leads to mitochondria collapse. Accumulation is increased in activated cells, making the effect of those rhodamines a particularly attractive therapy for activated cells in autoimmune diseases. Also, exposure of the immune system cells to other immunologic cells reacting toward host tissues or the transplanted organ, cancer cells, infected cells  
10 or other undesirable cells treated by PDT is particularly attractive for vaccination and extracorporeal photopheresis leading to beneficial immunomodulation.

PDT-treated cells and/or lysate thereof, including cell products released from these cells after PDT treatment with the photoactivatable molecules of the present invention, can be used either alone or in association with adjuvant in order  
15 to generate specific immune responses from an individual. These vaccines can be used to treat individuals suffering from autoimmune diseases such as, but not limited to: Rheumatoid Arthritis, Multiple Sclerosis, Scleroderma, Lupus erythematosus, Diabetes, Autoimmune Hemolytic Anemia, Diabetes Mellitus, Progressive Systemic Sclerosis, Idiopathic Thrombocytopenic Purpura, Psoriasis,  
20 Ulcerative Colitis, Crohn's Disease as well as to illnesses evading the immune system such as, but not limiting to: Acquired Immunodeficiency Syndrome (AIDS), Human Immunodeficiency Virus (HIV), Hepatitis C Virus (HCV), Human Herpes Virus Type I or II, and Varicella Zoster. Moreover, these vaccines can also lead to the improvement of cancer treatments by inducing an immune  
25 response to the evading cancer cells. This could lead to the physical destruction of tumor masses induced by a directed immune response.

In the present invention, the treatment of the individual cells is effected *ex vivo*, *in vitro* or *in vivo*. Preferably, the treatment is effected *ex vivo* by perfusion.  
30 Extracorporeal treatment of cells can also be used for the repetitive injection of a portion of PDT treated blood cells, which are then reinjected into the individuals.

- 20 -

This treatment is used for the improvement of acute and chronic conditions such as, but not limited to, Graft-versus-Host Disease, organ rejection, debilitating diseases caused by an autoimmune reaction such as, but not limited, to Rheumatoid Arthritis, Multiple Sclerosis, Scleroderma, Lupus, Type I and II  
5 Diabetes, Autoimmune Hemolytic Anemia, Diabetes Mellitus, Progressive Systemic Sclerosis, Idiopathic Thrombocytopenic Purpura, Psoriasis, Ulcerative Colitis and Crohn's Disease. This treatment also enhances the immune response against treated cells by the individual.

More specifically, in a preferred vaccine of the present invention, when  
10 PDT treated lymphoma cells or lymphoma cells supernatant obtained after exposure to PDT, are injected into mice, a significant decrease of lymphoma formation is observed. Such alleviation has been demonstrated using the T-cell lymphoma cell line EL-4. EL-4 lymphoma cells undergoing PDT with TH9402 and light rapidly proceed to programmed cell death, apoptosis and/or necrosis (see  
15 Example 1).

Repetitive subcutaneous injections of the supernatant from PDT treated EL-4 cells, or from irradiated PDT treated EL-4 cells in mice for 4 weeks followed by injection of non-treated cells clearly indicate that mice are demonstrating delayed growth of lymphoma. In contrast, non-vaccinated mice develop earlier  
20 lymphoma cell growth leading to death (Fig. 2).

Immunomodulation is believed to be performed through the unique potential of the immune system to develop an aggressive and specific response toward dysfunctional or dying cells. Antigen presenting cells process and present antigens based on their propensity to process antigens from cells undergoing  
25 programmed cell death or apoptosis. Since mainly activated cells will be eradicated by photoactivatable molecules of the present invention (TH9402 and derivatives thereof), analysis of the cell population undergoing apoptosis and necrosis has been evaluated. Data indicates that B-cells, NK cells, activated T-cells among others, are rapidly eliminated. This advantage is exploited by  
30 inducing the immune system to produce an immune response against autoreactive T-cells. This property has been used in mice models and humans developing

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GvHD. Peripheral blood cells from individuals with GvHD are harvested, usually by leukopheresis, and exposed to PDT. These treated cells are then reinfused into the individual and this procedure is repeated at regular intervals. This treatment leads to improvement of GvHD that occurs after stem cell transplantation. PDT using photoactivatable molecules of the present invention (TH9402 and derivatives thereof) is able to prevent the development or treat GvHD in mice that received PDT-treated cells at regular intervals. This leads to improved survival of mice infused with PDT-treated cells. In contrast, mice receiving either non-PDT treated cells or media alone are developing GvHD leading to death. This is also shown in Fig. 1 using Kaplan-Meier survival analysis.

The present invention will be more readily understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

#### Example 1

##### **Treatment of GvHD in mice**

One preferred embodiment of the present invention is to use whole cells exposed to photoactivatable molecules of the present invention (TH9402 and derivatives thereof) with PDT and also cell lysates generated after such treatment.

#### **Materials and Methods**

##### **Extracorporeal Phototherapy:**

**Mice.** The following strains of mice were purchased from The Jackson Laboratory: C57BL/6 (B6) (H-2<sup>b</sup>), B10BR (H-2<sup>k</sup>). Mice were bred and housed in specific pathogen-free conditions at the Guy-Bernier Research Centre according to the standards set by the Canadian Committee for Animal Protection. All mice were used between 6-10 weeks of age.

**Cell Transplantation.** Bone marrow cells were harvested from tibias and femurs of donor mice, T cell depleted and transplanted in recipient mice. Briefly, cells were suspended at a concentration of  $1 \times 10^7$  cells /ml in RPMI 1640 supplemented with 5% FBS, 100U/ml penicillin G, and 100µg/ml streptomycin, and incubated with rabbit anti-mouse T cells (Thy1) antiserum (Cedarlane Labs, Hornby,



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Ontario, Canada) at 4°C for 1 hour. The cells were then pelleted by centrifugation, resuspended in rabbit serum (Low-Tox-M rabbit complement; Cedarlane Labs.) as a source of complement, and incubated at 37°C for 1 hour. Cell suspensions were washed three times and analyzed for efficacy of depletion by flow cytometry using an anti-Thy1.2 Ab, and cell numbers adjusted for injection. Recipient mice received 1000 cGy total body irradiation from a <sup>60</sup>Co source at a dose rate of 128 cGy/minute on the day of transplant. Bone marrow and spleen cells were given as a single intravenous injection via the tail vein.

**Induction of GvHD.** GvHD was induced by intravenous injection of a suspension of 1.0 x 10<sup>6</sup> B6 (H-2<sup>b</sup>) splenocytes, along with the 1x10<sup>7</sup> T cell-depleted bone marrow cells described above into irradiated recipients: B10BR (H-2<sup>k</sup>; principal party) resulting in B6 x B10BR mice. B6 mice were also injected with 5x10<sup>6</sup> T cells for syngeneic controls.

**Photodynamic treatment.** For the purposes of the Kaplan-Meier analysis illustrated in Fig. 1, B10BR mice were first transplanted with bone marrow and splenocytes from B6 mice. Starting on day 14, some of these mice were sacrificed (B6 x B10BR) and their splenocytes (either PDT-treated or not) were administered to other B6 x B10BR mice. Splenocytes were obtained from animals that were transplanted in the above conditions. The cells were harvested, washed and resuspended at a density of 1x10<sup>6</sup> cells/ml in X-VIVO 15<sup>TM</sup> medium (Bio-Whittaker, Walkersville, MD) supplemented with 2.5% FBS. The cells were then allowed to internalize 10 µM TH9402 for 40 minutes. After a wash with X-VIVO 15 medium supplemented with 10% FBS, the photosensitizer was cleared from cells for 50 minutes. At the end of efflux time, the samples were submitted to photodynamic therapy with 5 J/cm<sup>2</sup> of light energy at a wavelength 514 nm, and using a sample thickness of 1.7 mm. Four million T cells of the PDT treated or PDT untreated group were injected into recipient mice at days 15, 22, 29, 36, 43 and 50 post-transplantation. As controls, one group of animals received only culture medium (RPMI-1640), and one group of syngeneic mice (B10BR (H-2k) in B10BR (H-2k)) received cells with or without PDT treatment on the same days as the GvHD groups. Cell administration was performed every week, starting on

- 23 -

day 14, for a total of 6 injections. Mice receiving PDT-treated cells had an improved survival over mice receiving cells that were not PDT-treated (Fig. 1). Treatment with PDT did not affect the survival of control mice receiving cells from syngeneic donors. In contrast, B10BR mice transplanted with B6 donor cells, and that received only medium, died rapidly.

### Example 2

#### **Tumor vaccination in mice**

The strain of mice B6SJL was used for the evaluation of PDT to induce immuno-protection. In generation of tumor cell lysates, EL-4 cells (American Type Culture Collection, ATCC Accession # TIB-39) were seeded in flasks at  $10^6$  cells/ml and exposed to  $10\mu\text{M}$  Th9402 in serum free DMEM without phenol red medium for 40 minutes, followed by exposure to drug-free medium for 90 minutes, then illuminated with a dose of  $10\text{J}/\text{cm}^2$ . Treated cells were incubated overnight. After incubation, cells and supernatants were collected and spun down. The resulting supernatant was collected, concentrated by vacuum speed using a molecular sieve (centriplus 3000 molecular weight cut-off), and stored frozen at  $-70^\circ\text{C}$  until use.

Six to eight-week old mice were vaccinated subcutaneously on the shoulder with  $40\mu\text{l}$  of either lysates or medium only once a week for 4 weeks. The animals were rested for a week and then inoculated on the flank with  $1-3 \times 10^4$  tumor cells. A medium alone (DMEM) group served as untreated control. Once the tumor cells were injected, tumor growth was monitored for 90 days. Animals immunized with the supernatant from PDT-treated cells had a delay in tumor cell appearance, in comparison to animals immunized with medium only (DMEM). The results are presented in Fig. 2. The data indicate that the supernatant from PDT treated cells delay the appearance of tumor compared to the medium control group. These results are in agreement with Korbelik et al (1996) in which they reported that PDT cell lysates following Photofrin treatment do induce a delayed tumor growth.

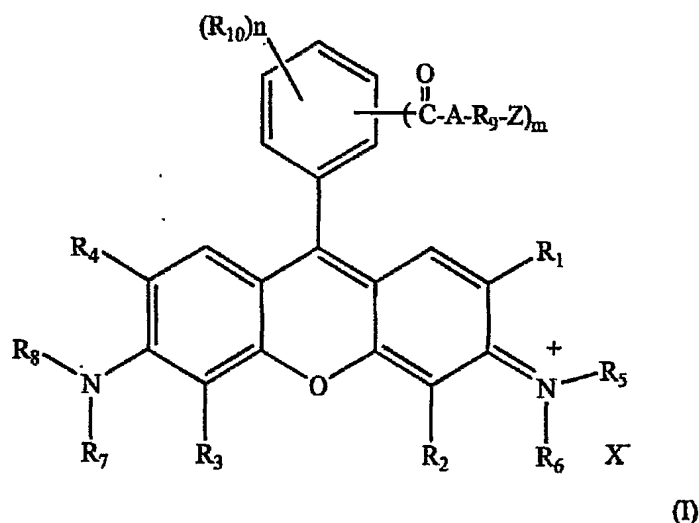
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While the invention has been described with particular reference to the illustrated embodiment, it will be understood that numerous modifications thereto will appear to those skilled in the art. Accordingly, the above description and accompanying drawings should be taken as illustrative of the invention and not in  
5 a limiting sense.

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**WHAT IS CLAIMED IS:**

1. The use of PDT-treated cells (whole or fragments thereof) and/or supernatant thereof in the preparation of an immunologic compound for prevention, protection, prophylaxis or treatment of an immunological disorder, infection and/or a cancer in an individual, which comprises treatment of said individual cells or components thereof with a photoactivatable molecule of formula (I):



wherein:

- 15 - one of R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, and R<sub>10</sub> represents an halogen atom and each of the remaining R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, and each of the remaining R<sub>10</sub> group is independently selected in the group constituted by hydrogen, halogen atoms, an amino, acylamino, dialkylamino, cycloalkylamino, azacycloalkyl, alkylcycloalkylamino, aroylamino, diarylamino, arylalkylamino, aralkylamino, alkylaralkylamino, arylaralkylamino, hydroxy, alkoxy, aryloxy, aralkyloxy, mercapto, alkylthio, arylthio, aralkylthio, carboxyl, alkoxycarbonyl, aryloxycarbonyl, aralkoxycarbonyl, carbamoyl, alkylcarbamoyl, dialkylcarbamoyl, cyano, hydroxysulfonyl, amidosulfonyl, dialkylamidofulfonyl,
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arylalkylamidossulfonyl, formyl, acyl, aroyl, alkyl, alkylene, alkenyl, aryl, aralkyl, vinyl, alkynyl group and by the corresponding substituted groups;

- m = 0 - 1;

5

- n = 1-4;

- A is nil, O, or NH;

- R<sub>9</sub> represents an alkylene group;

- Z is H, amino, dialkylamino, or trialkylamino salt;

- X is an anion; and

10

- R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub> and R<sub>8</sub> are independently H or C<sub>1</sub>-C<sub>6</sub> alkyl or R<sub>1</sub> in combination with R<sub>5</sub> or R<sub>6</sub>, or R<sub>2</sub> in combination with R<sub>5</sub> or R<sub>6</sub>, or R<sub>3</sub> in combination with R<sub>7</sub> or R<sub>8</sub>, or R<sub>4</sub> in combination with R<sub>7</sub> or R<sub>8</sub> represents an alkylene,

and wherein said photoactivatable molecule is activated by a light of appropriate wavelength, thereby activating said photoactivatable molecule and causing prevention, protection, prophylaxis or treatment of said immunological disorder, infection and/or a cancer.

2. The use of claim 1, wherein said immunologic compound is a vaccine.

20

3. The use of claim 1 or 2, wherein said immunological disorder is an alloimmune disorder or an autoimmune disorder.

4. The use of claim 3, wherein said alloimmune disorder is Graft-versus-Host Disease or an organ rejection.

5. The use of claim 3, wherein said autoimmune disease is selected from the group consisting of Rheumatoid Arthritis, Multiple Sclerosis, Scleroderma, Lupus, Autoimmune Hemolytic Anemia, Diabetes Mellitus, Progressive Systemic Sclerosis, Idiopathic Thrombocytopenic Purpura, Psoriasis, Ulcerative Colitis and Crohn's Disease.

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6. The use of any one of claims 1 to 5, wherein said infection is caused by a bacteria, a virus, a parasite, a fungus, a prion or a protozoan.
- 5 7. The use of claim 6, wherein said virus is selected from the group consisting of Human Immunodeficiency Virus (HIV), Hepatitis C Virus (HCV), Human Herpes Virus Type I or II, and Varicella Zoster.
8. The use of any one of claims 1 to 7, wherein said infection causes  
10 Chagas' Disease.
9. The use of any one of claims 1 to 8, wherein said cancer is selected from the group consisting of solid tumors and hematologic tumors.
- 15 10. The use of claim 9, wherein said solid tumors are of breast cancer, lung cancer, gastrointestinal cancer, skin cancer or of genitourinary, neurological, head and neck or musculoskeletal origin.
11. The use of claim 9, wherein said hematologic tumors are lymphomas,  
20 leukemias, myelomas, myelodysplasias or plasma cell dyscrasias.
12. The use of any one of claims 1 to 11, wherein said treatment of said individual cells is effected *ex vivo*, *in vitro* or *in vivo*.
- 25 13. The use of claim 12, wherein said treatment is an *ex vivo* treatment effected by perfusion.
14. The use of any one of claims 1 to 13, wherein said photoactivatable molecule is selected from the group consisting of:  
30 4,5-dibromorhodamine 123 hydrobromide (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)-benzoic acid methyl ester hydrobromide) also called TH9402,

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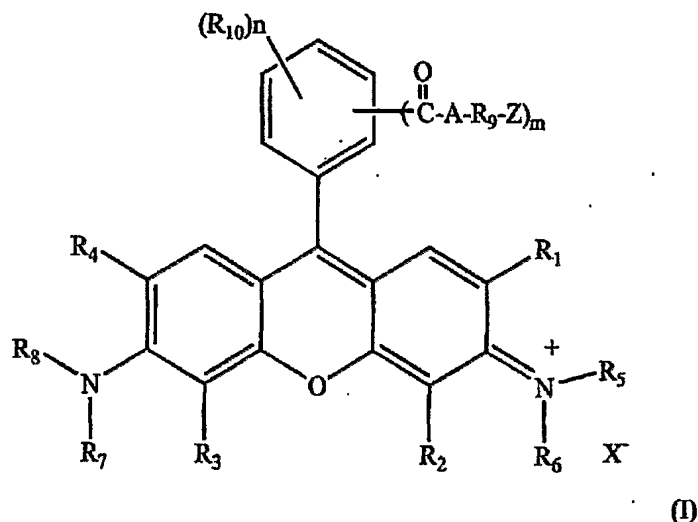
- 4,5-dibromorhodamine 123 hydrochloride (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)-benzoic acid methyl ester hydrochloride)
- 5 4,5-dibromorhodamine 110 ethyl ester hydrochloride (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid ethyl ester hydrochloride),
- 10 4,5-dibromorhodamine 110 octyl ester hydrochloride (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid octyl ester hydrochloride),
- 15 4,5-dibromorhodamine 110 n-butyl ester hydrochloride (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid n-butyl ester hydrochloride),
- 20 rhodamine B n-butyl ester hydrochloride (2'-(6-diethyl amino-3-diethyl imino-3H-xanthen-9-yl)benzoic acid n-butyl ester hydrochloride),
- 25 4,5-dibromorhodamine 110 ethyl ester hydrobromide (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid ethyl ester hydrobromide),
- 30 4,5-dibromorhodamine 110 octyl ester hydrobromide (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid octyl ester hydrobromide),
- 35 4,5-dibromorhodamine 110 n-butyl ester hydrobromide (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid n-butyl ester hydrobromide),
- 4',5'-dichlorotetramethylrhodamine (2'-(6-dimethylamino-3-dimethylimino-3H-xanthen-9-yl)-4',5'-dichloro benzoic acid methyl ester hydrochloride),
- 4,5-dibromorhodamine 110 2-(2-methoxy ethoxy)ethyl ester hydrobromide (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)-benzoic acid 2-(2-methoxy ethoxy) ethyl ester hydrobromide),
- 2,7-dibromorhodamine B hexyl ester acetate (2'-(2,7-dibromo-6-diethyl amino-3-diethyl imino-3H-xanthen-9-yl)benzoic acid hexyl ester acetate),

- 29 -

- 2,7-dibromorhodamine B methyl ester acetate (2'-(2,7-dibromo-6-diethyl amino-3-diethyl imino-3H-xanthen-9-yl)benzoic acid methyl ester acetate),
- 5 4,5-dibromorhodamine 6G hydrobromide (2'-(4,5-dibromo-2,7-dimethyl-6-ethylamino-3-ethylimino-3H-xanthen-9-yl)benzoic acid ethyl ester hydrobromide),
- rhodamine B 3-bromopropylester hydrochloride (2'-(6-diethyl amino-3-diethyl imino-3H-xanthen-9-yl)benzoic acid 3-bromopropyl ester hydrochloride),
- 10 4,5-dibromorhodamine B base (3,3-(4',5'-dibromo-3'-diethyl amino-6'-diethyl aminoxanthen-9'-yl)-3H-isobenzofuran-1-one),
- 2,7-dibromorhodamine B base (3,3-(2',7'-dibromo-3'-diethyl amino-6'-diethyl aminoxanthen-9'-yl)-3H-isobenzofuran-1-one) and
- 15 4-bromo-7-phenyl-rhodamine B base (3,3-(4'-bromo-3'-diethyl amino-6'-diethyl amino-5'-phenyl xanthen-9'-yl)-3H-isobenzofuran-1-one).
- 15 The use of any one of claims 1 to 14, wherein said wavelength is ranging from about 400 to about 800 nm.
- 20 16. The use of claim 15, wherein said wavelength is ranging from about 450 to about 600 nm.
17. An immunologic vaccine comprising PDT-treated cells (whole or
- 25 fragments thereof) and/or supernatant thereof, wherein said cells are treated with a photoactivatable molecule of formula (I):



- 30 -



wherein:

- 5       - one of  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ , and  $R_{10}$  represents an halogen atom and each of the remaining  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ , and each of the remaining  $R_{10}$  group is independently selected in the group constituted by hydrogen, halogen atoms, an amino, acylamino, dialkylamino, cycloalkylamino, azacycloalkyl, alkylcycloalkylamino, aroylamino, diarylamino, arylalkylamino, aralkylamino, alkylaralkylamino, arylaralkylamino,
- 10       hydroxy, alkoxy, aryloxy, aralkyloxy, mercapto, alkylthio, arylthio, aralkylthio, carboxyl, alkoxycarbonyl, aryloxycarbonyl, aralkoxycarbonyl, carbamoyl, alkylcarbamoyl, dialkylcarbamoyl, cyano, hydroxysulfonyl, amidosulfonyl, dialkylamidofulfonyl, arylalkylamidofulfonyl, formyl, acyl, aroyl, alkyl, alkylene, alkenyl,
- 15       aryl, aralkyl, vinyl, alkynyl group and by the corresponding substituted groups;
- $m = 0 - 1$ ;
- $n = 1 - 4$ ;
- A is nil, O, or NH;
- 20       -  $R_9$  represents an alkylene group;
- Z is H, amino, dialkylamino, or trialkylamino salt;
- $X^-$  is an anion; and

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- R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub> and R<sub>8</sub> are independently H or C<sub>1</sub>-C<sub>6</sub> alkyl or R<sub>1</sub> in combination with R<sub>5</sub> or R<sub>6</sub>, or R<sub>2</sub> in combination with R<sub>5</sub> or R<sub>6</sub>, or R<sub>3</sub> in combination with R<sub>7</sub> or R<sub>8</sub>, or R<sub>4</sub> in combination with R<sub>7</sub> or R<sub>8</sub> represents an alkylene,

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in association with a pharmaceutically acceptable carrier.

18. The vaccine of claim 17, wherein said photoactivatable molecule is selected from the group consisting of

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4,5-dibromorhodamine 123 hydrobromide (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)-benzoic acid methyl ester hydrobromide) also called TH9402,

15

4,5-dibromorhodamine 123 hydrochloride (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)-benzoic acid methyl ester hydrochloride)

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4,5-dibromorhodamine 110 ethyl ester hydrochloride (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid ethyl ester hydrochloride),

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4,5-dibromorhodamine 110 octyl ester hydrochloride (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid octyl ester hydrochloride),

4,5-dibromorhodamine 110 n-butyl ester hydrochloride (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid n-butyl ester hydrochloride),

30

rhodamine B n-butyl ester hydrochloride (2'-(6-diethyl amino-3-diethyl imino-3H-xanthen-9-yl)benzoic acid n-butyl ester hydrochloride),

35

4,5-dibromorhodamine 110 ethyl ester hydrobromide (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid ethyl ester hydrobromide),

4,5-dibromorhodamine 110 octyl ester hydrobromide (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid octyl ester hydrobromide),

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- 4,5-dibromorhodamine 110 n-butyl ester hydrobromide (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid n-butyl ester hydrobromide),
- 5 4',5'-dichlorotetramethylrhodamine (2'-(6-dimethylamino-3-dimethylimino-3H-xanthen-9-yl)-4',5'-dichloro benzoic acid methyl ester hydrochloride),
- 4,5-dibromorhodamine 110 2-(2-methoxy ethoxy)ethyl ester hydrobromide (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)-benzoic acid 2-(2-methoxy ethoxy) ethyl ester hydrobromide),
- 10 2,7-dibromorhodamine B hexyl ester acetate (2'-(2,7-dibromo-6-diethyl amino-3-diethyl imino-3H-xanthen-9-yl)benzoic acid hexyl ester acetate),
- 15 2,7-dibromorhodamine B methyl ester acetate (2'-(2,7-dibromo-6-diethyl amino-3-diethyl imino-3H-xanthen 9-yl)benzoic acid methyl ester acetate),
- 4,5-dibromorhodamine 6G hydrobromide (2'-(4,5-dibromo-2,7-dimethyl-6-ethylamino-3-ethylimino-3H-xanthen-9-yl)benzoic acid ethyl ester hydrobromide),
- 20 rhodamine B 3-bromopropylester hydrochloride (2'-(6-diethyl amino-3-diethyl imino-3H-xanthen-9-yl)benzoic acid 3-bromopropyl ester hydrochloride),
- 4,5-dibromorhodamine B base (3,3-(4',5'-dibromo-3'-diethyl amino-6'-diethyl aminoxanthen-9'-yl)-3H-isobenzofuran-1-one),
- 25 2,7-dibromorhodamine B base (3,3-(2',7'-dibromo-3'-diethyl amino-6'-diethyl aminoxanthen-9'-yl)-3H-isobenzofuran-1-one) and
- 4-bromo-7-phenyl-rhodamine B base (3,3-(4'-bromo-3'-diethyl amino-6'-diethyl amino-5'-phenyl xanthen-9'-yl)-3H-isobenzofuran-1-one).
- 30

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19. The vaccine of claim 17 or 18, wherein said molecule is acitivatable by a light having a wavelength ranging from about 400 to about 800 nm.
20. The vaccine of claim 19, wherein said wavelength ranges from about  
5 450 to about 600 nm.
21. The use of a vaccine as defined in any one of claims 17 to 20 for prevention, protection, prophylaxis or treatment of an immunological disorder, infection and/or a cancer.  
10
22. The use of claim 21, wherein said immunological disorder is an alloimmune disorder or an autoimmune disorder.
23. The use of claim 22, wherein said alloimmune disorder is Graft-versus-  
15 Host Disease or an organ rejection.
24. The use of claim 22, wherein said autoimmune disease is selected from the group consisting of Rheumatoid Arthritis, Multiple Sclerosis, Scleroderma, Lupus, Autoimmune Hemolytic Anemia, Diabetes Mellitus, Progressive Systemic  
20 Sclerosis, Idiopathic Thrombocytopenic Purpura, Psoriasis, Ulcerative Colitis and Crohn's Disease.
25. The use of any one of claims 21 to 24, wherein said infection is caused by a bacteria, a virus, a parasite, a fungus, a prion or a protozoan.  
25
26. The use of claim 25, wherein said virus is selected from the group consisting of Human Immunodeficiency Virus (HIV), Hepatitis C Virus (HCV), Human Herpes Virus Type I or II, and Varicella Zoster.
- 30 27. The use of any one of claims 21 to 26, wherein said infection causes Chagas' Disease.
28. The use of any one of claims 21 to 27, wherein said cancer is selected from the group consisting of solid tumors and hematologic tumors.  
35

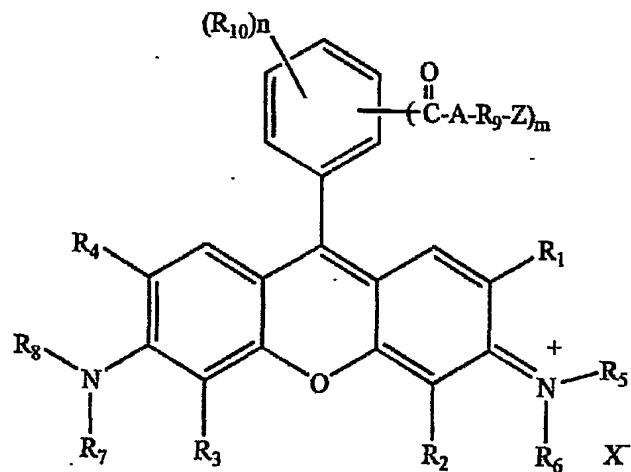
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29. The use of claim 28, wherein said solid tumors are of breast cancer, lung cancer, gastrointestinal cancer, skin cancer or of genitourinary, neurological, head and neck or musculoskeletal origin.

5 30. The use of claim 28, wherein said hematologic tumors are lymphomas, leukemias, myelomas, myelodysplasias or plasma cell dyscrasias.

31. A method of preparing an immunologic compound for prevention,  
10 protection, prophylaxis or treatment of an immunological disorder, infection and/or a cancer in an individual, which comprises the steps of:

a) treatment of said individual cells with a photoactivatable molecule of formula (I):



(I)

wherein:

20 - one of  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ , and  $R_{10}$  represents an halogen atom and each of the remaining  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ , and each of the remaining  $R_{10}$  group is independently selected in the group constituted by hydrogen, halogen atoms, an amino, acylamino, dialkylamino, cycloalkylamino, azacycloalkyl, alkylcycloalkylamino, aroylamino, diarylamino, arylalkylamino, aralkylamino, alkylaralkylamino, arylaralkylamino,

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hydroxy, alkoxy, aryloxy, aralkyloxy, mercapto, alkylthio, arylthio, aralkylthio, carboxyl, alkoxycarbonyl, aryloxy carbonyl, aralkoxycarbonyl, carbamoyl, alkylcarbamoyl, dialkylcarbamoyl, cyano, hydroxysulfonyl, amidosulfonyl, dialkylamid sulfonyl, arylalkylamid sulfonyl, formyl, acyl, aroyl, alkyl, alkylene, alkenyl, aryl, aralkyl, vinyl, alkynyl group and by the corresponding substituted groups;

- m = 0 - 1;

- n = 1-4;

- A is nil, O, or NH;

- R<sub>9</sub> represents an alkylene group;

- Z is H, amino, dialkylamino, or trialkylamino salt;

- X is an anion; and

- R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub> and R<sub>8</sub> are independently H or C<sub>1</sub>-C<sub>6</sub> alkyl or R<sub>1</sub> in combination with R<sub>5</sub> or R<sub>6</sub>, or R<sub>2</sub> in combination with R<sub>5</sub> or R<sub>6</sub>, or R<sub>3</sub> in combination with R<sub>7</sub> or R<sub>8</sub>, or R<sub>4</sub> in combination with R<sub>7</sub> or R<sub>8</sub> represents an alkylene,

and

b) subjecting said cells to a light of appropriate wavelength to activate said photoactivatable molecule, thereby obtaining PDT-treated individual cells (whole or fragments thereof) and/or supernatant thereof.

32. The method of claim 31, wherein said immunologic compound is an autoimmune vaccine.

33. The method of claim 31 or 32, wherein said immunological disorder is an alloimmune disorder or an autoimmune disorder.

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34. The method of claim 33, wherein said alloimmune disorder is Graft-versus-Host Disease or an organ rejection.

35. The method of claim 33, wherein said autoimmune disease is selected  
5 from the group consisting of Rheumatoid Arthritis, Multiple Sclerosis, Scleroderma, Lupus, Autoimmune Hemolytic Anemia, Diabetes Mellitus, Progressive Systemic Sclerosis, Idiopathic Thrombocytopenic Purpura, Psoriasis, Ulcerative Colitis and Crohn's Disease.

10 36. The method of any one of claims 31 to 35, wherein said infection is caused by a bacteria, a virus, a parasite, a fungus, a prion or a protozoan.

37. The method of claim 36, wherein said virus is selected from the group consisting of Human Immunodeficiency Virus (HIV), Hepatitis C Virus (HCV),  
15 Human Herpes Virus Type I or II, and Varicella Zoster.

38. The method of any one of claims 31 to 37, wherein said infection causes Chagas' Disease.

20 39. The method of any one of claims 31 to 38, wherein said cancer is selected from the group consisting of solid tumors and hematologic tumors.

40. The method of claim 39, wherein said solid tumors are of breast cancer, lung cancer, gastrointestinal cancer, skin cancer or of genitourinary, neurological,  
25 head and neck or musculoskeletal origin.

41. The method of claim 39, wherein said hematologic tumors are lymphomas, leukemias, myelomas, myelodysplasias or plasma cell dyscrasias.

30 42. The method of any one of claims 31 to 41, wherein said treatment of said individual cells is effected *ex vivo*, *in vitro* or *in vivo*.

43. The method of claim 42, wherein said treatment is an *ex vivo* treatment effected by perfusion.

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44. The method of any one of claims 31 to 43, wherein said photoactivatable molecule is selected from the group consisting of :

5 4,5-dibromorhodamine 123 hydrobromide (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)-benzoic acid methyl ester hydrobromide) also called TH9402,

10 4,5-dibromorhodamine 123 hydrochloride (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)-benzoic acid methyl ester hydrochloride)

4,5-dibromorhodamine 110 ethyl ester hydrochloride (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid ethyl ester hydrochloride),

15 4,5-dibromorhodamine 110 octyl ester hydrochloride (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid octyl ester hydrochloride),

20 4,5-dibromorhodamine 110 n-butyl ester hydrochloride (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid n-butyl ester hydrochloride),

rhodamine B n-butyl ester hydrochloride (2'-(6-diethyl amino-3-diethyl imino-3H-xanthen-9-yl)benzoic acid n-butyl ester hydrochloride),

25 4,5-dibromorhodamine 110 ethyl ester hydrobromide (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid ethyl ester hydrobromide),

30 4,5-dibromorhodamine 110 octyl ester hydrobromide (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid octyl ester hydrobromide),

4,5-dibromorhodamine 110 n-butyl ester hydrobromide (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)benzoic acid n-butyl ester hydrobromide),

35 4',5'-dichlorotetramethylrhodamine (2'-(6-dimethylamino-3-dimethylimino-3H-xanthen-9-yl)-4',5'-dichloro benzoic acid methyl ester hydrochloride),



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- 4,5-dibromorhodamine 110 2-(2-methoxy ethoxy)ethyl ester hydrobromide (2'-(6-amino-4,5-dibromo-3-imino-3H-xanthen-9-yl)-benzoic acid 2-(2-methoxy ethoxy) ethyl ester hydrobromide),
- 5 2,7-dibromorhodamine B hexyl ester acetate (2'-(2,7-dibromo-6-diethyl amino-3-diethyl imino-3H-xanthen-9-yl)benzoic acid hexyl ester acetate),
- 10 2,7-dibromorhodamine B methyl ester acetate (2'-(2,7-dibromo-6-diethyl amino-3-diethyl imino-3H-xanthen 9-yl)benzoic acid methyl ester acetate),
- 4,5-dibromorhodamine 6G hydrobromide (2'-(4,5-dibromo-2,7-dimethyl-6-ethylamino-3-ethylimino-3H-xanthen-9-yl)benzoic acid ethyl ester hydrobromide),
- 15 rhodamine B 3-bromopropylester hydrochloride (2'-(6-diethyl amino-3-diethyl imino-3H-xanthen-9-yl)benzoic acid 3-bromopropyl ester hydrochloride),
- 4,5-dibromorhodamine B base (3,3-(4',5'-dibromo-3'-diethyl amino-6'-diethyl aminoxanthen-9'-yl)-3H-isobenzofuran-1-one),
- 20 2,7-dibromorhodamine B base (3,3-(2',7'-dibromo-3'-diethyl amino-6'-diethyl aminoxanthen-9'-yl)-3H-isobenzofuran-1-one) and
- 4-bromo-7-phenyl-rhodamine B base (3,3-(4'-bromo-3'-diethyl amino-6'-diethyl amino-5'-phenyl xanthen-9'-yl)-3H-isobenzofuran-1-one).
- 25 45. The method of any one of claims 31 to 44, wherein said wavelength is ranging from about 400 to about 800 nm.
46. The method of claim 45, wherein said wavelength is ranging from about 450 to about 600 nm.

**Figure 1: Cumulative proportion (Kaplan-Meier) of mice surviving after administration of cells treated or not with PDT**

